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Analysis of Polychlorinated Biphenyls (PCBs) in Human Serum

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Polychlorinated biphenyls (PCBs) are widely dispersed in the environment. Occupational exposure to PCBs takes place, e.g., in manufacture and repair of capacitors and transformers. A special type of exposure of workers to PCBs may occur in connection with accidents where capacitors or transformers containing PCBs products are damaged, as in the case of a fire or electric arcing.

The quantitation of PCBs poses several problems: There are 209 different homologs and isomers of PCBs. The isomers of PCBs have different responses in the electron capture detector, and the pattern of PCBs in biological specimens is different from that of commercial products.

In this study a method was worked out for the analysis of PCBs in serum with the purpose of estimating occupation exposure to PCBs. The PCBs were extracted to an organic solvent, and the extract was purified by use of sulfuric acid and silica columns. Capillary gas chromatography with electron capture detection was used. For separation, temperature programming was used.

Introduction

Polychlorinated biphenyls (PCBs) are widely dispersed in the environment. Because of their great stability and lipid solubility they accumulate in animal tissues. These compounds are found at every level of the food chain. Therefore, all men are exposed to PCBs. In Finland, the main source of PCBs is fish, the daily dose of PCBs for an average Finn has been estimated to be 7 μg (*1*). The heaviest occupational exposure to PCBs occurs in manufacture and repair of capacitors and transformers. A special type of exposure of workers to PCBs may occur in connection of accidents where capacitors or transformers containing PCB products are damaged, as in the case of a fire or electric arcing. However, there is no literature on the uptake of PCBs in these incidents. In these instances the toxicological interest is mainly in exposure to pyrosynthetic derivatives of PCBs; increased levels of PCBs in serum may indicate only that exposure has taken place.

There are, in all, 209 different homologs and isomers of PCBs; 40 to 80 main components may be detected in commercial PCB mixtures. There are several commercial PCB products: Clophen A30, A50, A60, Aroclor 1224, 1232, 1264, etc., with different levels of chlorination (*2,3*) and different isomer compositions. Therefore the quantitation of PCB poses formidable problems.

An additional problem in the quantitation is the different response of the electron capture detector to different isomers. In biological specimens the peak pattern usually differs from that of commercial products because different isomers behave differently in the organism. Therefore, use as a standard of typical commercial products traditionally used (*4–10*) in PCB analysis gives erroneous results in biological samples.

In the present paper we describe an analytical method for serum PCB, in which pure isomers are used for the quantitation. the extraction and purification procedures have been optimized for serum analysis. The reference values for nonexposed Finns have been determined. The method has also been applied to analysis of exposure of workers to PCBs in capacitor accidents.

Subjects

For the determination of the reference interval, venous blood specimens were collected after an overnight fast from workers of metal industry and forestry with no known exposure to PCBs. Serum was separated by centrifugation and stored at –20°C or –70°C until analyzed. The specimens from workers with potential exposure to PCBs and their pyrosynthetic derivatives in capacitor accidents were collected and treated similarly.

Material and Methods

Apparatus

A Hewlett-Packard 5880A gas–liquid chromatograph (GLC) equipped with a Hewlett-Packard model 7672 A

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automatic sampler and an ^{63}Ni electron capture detector (ECD) were used. The chromatograph was controlled and the data were calculated by Hewlett-Packard Level IV terminal. A 25 m OV-1701 silica-capillary (phase 0.25 μm, Orion Analytica, Finland) column was used. The oven temperature was programmed from 80°C to 180°C at 30°C/min, 180°C to 230°C at 5°C/min, 7 min at 230°C. The injector was kept at 250°C and the detector at 350°C. Carrier gas (He) flow was 1 mL/min, argon-methane (5%) was used as the make-up gas with a flow of 30 mL/min. Splitless injection was used.

Bond Elut (Analytichem International, Harbor City, USA) commercial silica columns were used for the clean up together with the reduced pressure-elutor Vac-Elut (Analytichem International, Harbor City, USA). Mixing for the extraction was achieved by a Heidolph (Heidolph-Elektra KG, Kelheim, FRG) mechanical shaker.

Reagents

Methanol, *n*-hexane (Rathburn, Walkerburn, Scotland), and diethyl ether (anhydrous, J. T. Baker, Deventer, The Netherlands) were of HPLC-grade. Acetone (p.a.), Na_2SO_4 (p.a.) and H_2SO_4 (suprapur) were purchased from Merck (Karmstadt, FRG).

Standards

2,4′-Di-, 2,5,2′-tri-, 2,4,4′-tri-, 3,4,2′-tri-, 2,4,2′,4′-tetra-, 2,3,2′,5′-tetra-, 2,4,3′,4′-tetra-, 3,4,3′,4′-tetra, 2,4,6,3′,5′-penta-, 2,4,5,2′,5′-penta-, 2,4,5,2′,3′-penta- and 2,3,4,2′,5′-pentachlorobiphenyl were purchased from Analabs (North Haven, CT). The 2,2′- and 4,4′-dichlorobiphenyls were also tested, but the response of the EC detector toward them was very poor (see Table 1); they could not be found in serum.

For the primary standard solutions a 1 to 4 mg portion of each isomer was weighed and dissolved in *n*-hexane. Further diluted solutions of different isomers were then combined (in acetone) and further diluted (with hexane or serum) to give the final standards. The concentration of the different isomers in the final standards was from 1.5 to 3.5 μg/L (lowest) to 7.5 to 17.5 μg/L (highest). Five standards with different concentration levels were used in every series.

Internal Standards

For use as internal standards, two asymmetrical PCBs isomers, 2,4,6-trichlorobiphenyl and 2,3,4,5,6-pentachlorobiphenyl were dissolved in *n*-hexane to give a final concentration of 6 to 10 μg/L.

Table 1. PCB isomers, their order of elution, correlation coefficients (*r*, range) of the standard curves and EC detection responses.

Analabs RCS-code	PCB isomer	rt/rt_{ISTD}	*r*, range (N = 20–31)	Proportional (EC) responses 3,3′ = 1.0
012	2,6	0.495		7.0
005	2,2′	0.495		1.8
010	2,4	0.495		11.4
011	2,5	0.495		6.8
008	2,4′	0.527	0.961–1.000	6.6
009	2,3	0.528		8.8
014	3,5	0.535		3.0
023	2,4,6	0.544		21.2
006	3,3′	0.586		1.0
016	2,5,2′	0.588	0.972–1.000	7.4
013	3,4	0.593		3.6
021	2,3,6	0.602		2.2
007	4,4′	0.608		1.8
022	2,4,5	0.628		15.2
027	2,6,2′,6′	0.652		5.6
017	2,5,3′	0.653		10.2
015	2,4,4′	0.674	0.974–1.000	15.4
018	2,5,4′	0.667		12.4
020	2,3,4	0.689		16.0
019	3,4,2′	0.701	0.982–1.000	9.6
026	2,5,2′,5′	0.737		10.6
030	2,4,2′,5′	0.742		13.8
025	2,4,2′,4′	0.748	0.977–1.000	15.4
033	2,3,5,6	0.744		30.8
029	2,3,2′,5	0.798	0.981–1.000	13.8
032	2,3,4,5	0.856		23.0
024	2,3,2′,3′	0.858		32.6
166	2,4,6,3′,5′	0.866	0.963–1.000	51.2
031	2,4,3′,4′	0.890		10.2
052	2,4,3′,4′	0.901	0.976–1.000	10.8
038	2,4,5,2′,5′	0.942	0.969–1.000	18.8
039	2,3,4,5,6	1.000		31.0
036	2,4,5,2′,3′	1.014	0.961–1.000	21.4
034	2,3,4,2′,5′	1.033	0.977–1.00	20.4
028	3,4,3′,4′	1.108	0.962–1.000	2.8
PST 250	(99%) pp′DDE	1.041		39.0

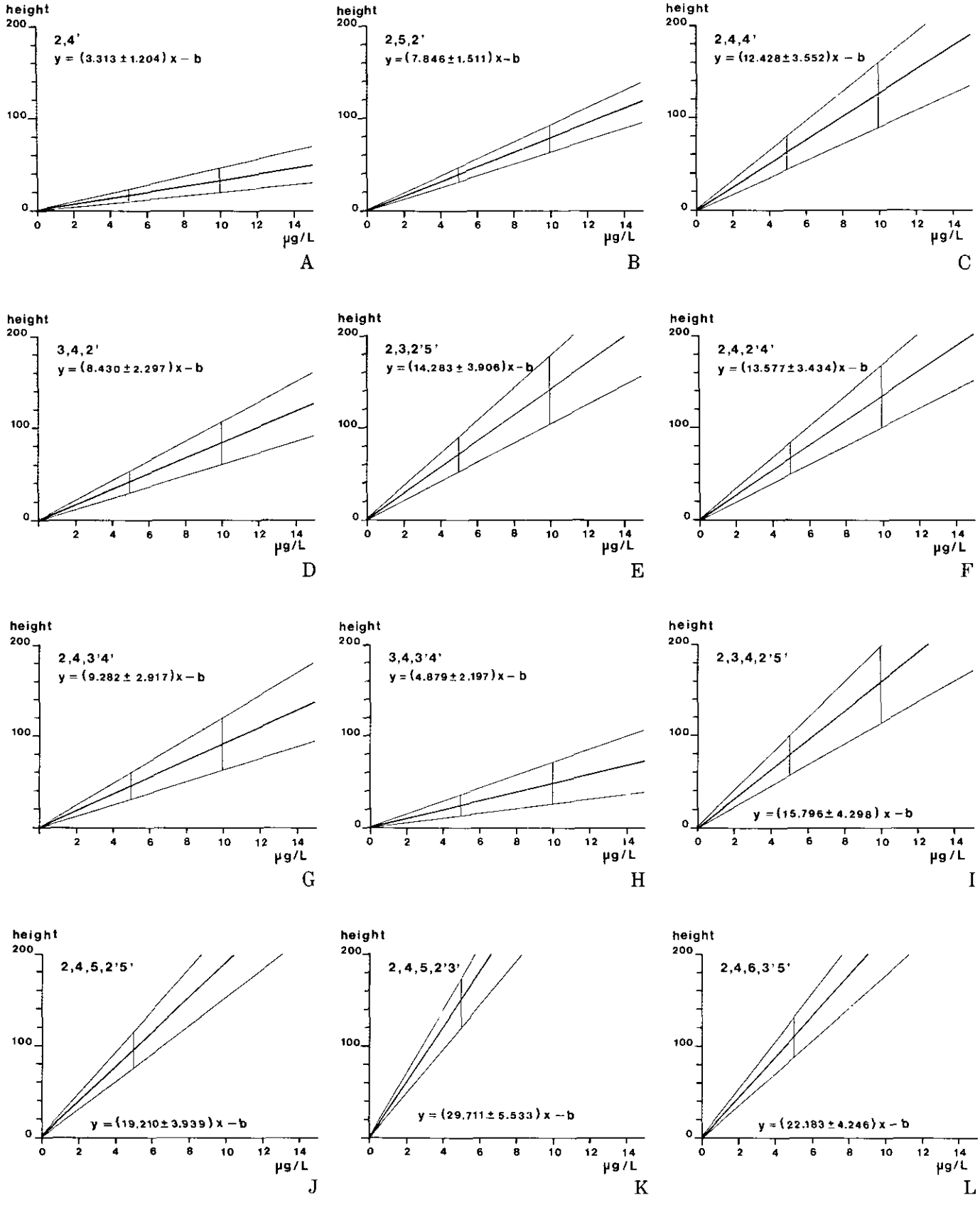

FIGURE 1. Peak height-concentration relationship of the individual PCBs isomers (means ± S.D. of the slopes are indicated, N = 12–21). For the correlation coefficients, see Table 1.

Extraction and Clean-up

All analyses were performed in duplicate. A 50-μL portion of the internal standard solution, 2 mL of methanol and 2 mL of the serum specimen were added successively to test tubes. The tubes were mixed in a mechanical shaker for 2 to 3 min. The serum–methanol mixture was extracted with 6 mL *n*-hexane: diethyl ether (1:1, vol/vol) with continuous mixing for 30 min. The layers were separated by centrifugation (2000 rpm, 10 min): 4 mL to the organic phase was concentrated under a gentle stream of nitrogen at room temperature to 2 mL. The concentrated organic phase was transferred to 2.5 mL of concentrated sulfuric acid and mixed well (by hand; a mixer must not be used). After separation of the phases by centrifugation (2000 rpm, 10 min), the organic layer was transferred to a tube containing 0.5 g Na_2SO_4 and mixed thoroughly to remove water from the solution. The organic phase after separation (2000 rpm, 10 min) was poured on Bond Elut silica columns prewashed with 15 to 20 mL of hexane.

The effluent was collected and the columns were rinsed with 2 mL of *n*-hexane (all combined for the next step). The eluate was concentrated under a gentle stream of nitrogen at room temperature to approximately 50 to 100 μL. The final concentrate was transferred to autosampler vials for the analysis.

Quantitation

The calculations are based on separate quantitation of peaks coeluting with the 12 standards and thus relies on the isomer-specific responses of the EC detector. Each peak is identified by its retention index (the proportion: $rt_{peak}/rt_{2,3,4,5,6\text{-PCB}}$). The width of the retention time window used is ± 0.5%. Each peak is then quantified separately using the peak heights. A standard curve is prepared for each isomer using five different concentration levels. A blank test value (Milli Q-distilled water instead of serum) is subtracted from the peak height of both samples and standards. A linear regression between peak height/internal standard ratio and the concentration of the standard in question is calculated with the method of least squares. The correlation coefficient (r) of the regression is usually better than 0.96 (see Fig. 1). The concentration of a single isomer (x_i) is calculated separately from

$$x_i = y_i VR/a$$

where y_1 = the peak height/internal standard height ratio, a = slope of the standard curve, V = volume correction factor (1/vol serum,mL), R = recovery (see below).

The final result is the sum of the isomer concentrations. Because the standards are prepared in hexane, the use of the internal standard does not correct the results for defective recovery of the extraction. Therefore, the recoveries of the PCBs have been determined by comparing standards in serum to standards in hexane. The recoveries of different isomers at different concentration levels are given in Table 2. Within every run it is verified that the recovery has remained the same, using one concentration level.

For internal quality control purposes, three different control specimens have been included in every series: control 0, pooled serum from a clinical laboratory; control 1, serum spiked with pure isomers at a level of about 4 to 6 μg/L; control 2, serum spiked to a concentration of approximately 20 μg/L.

Decontamination of Glassware

All the glassware used in this study was thoroughly rinsed first with hexane and then with acetone, after which they were subjected to normal glassware washing procedures.

Results and Discussion

During the development of this method every step in the procedure presented here was tested. First we tested a few methods published for PCB analysis (*11–13*). We were not, however, able to obtain good quality chromatograms with our capillary column with these meth-

Table 2. Recovery of the individual PCB isomers at five concentration levels.

Isomer	rt/rt_{ISTD} x	rt/rt_{ISTD} CV%	Total recovery % N	Total recovery % x±SD	Concn. level 1, μg/L	Recoveries of the five different concentration levels, x±SD Level 1	Level 2	Level 3	Level 4	Level 5
2,4′	0.527	0.2	20	84.8±19.2	2.6	78.8±27.7	81.7±16.9	81.4±13.3	90.9±19.9	91.2±18.3
2,5,2′	0.588	0.4	19	66.2±17.3	2.6	59.2±17.3	66.2±21.7	63.3±14.0	69.6±14.3	72.6±19.0
2,4,4′	0674	0.2	20	94.0±16.5	2.0	86.0±20.5	90.5±17.8	92.0±15.7	100.3±15.9	101.2±12.8
3,4,2′	0.701	0.2	19	83.0±15.1	2.0	73.8±15.5	81.9±17.0	81.3±14.0	87.2±14.0	90.8±15.2
2,4,2′,4′	0.748	0.1	19	80.3±13.0	2.6	76.0±12.7	80.3±12.3	79.2±12.3	82.5±13.8	83.7±13.8
2,3,2′,5′	0.798	0.1	19	76.6±15.0	1.9	70.0±20.5	77.1±16.8	74.2±13.2	79.3± 8.8	82.4±15.7
2,3,3′,4′	0.901	0.1	20	91.9±14.7	2.1	80.3±15.4	91.4±17.7	92.5±17.9	92.2±11.0	103.2±11.6
3,4,3′,4′	1.108	0.3	19	87.7±22.0	1.9	74.5±23.2	92.5±22.4	89.8±26.8	91.0±18.7	90.7±18.7
2,4,6,3′,5′	0.866	0.1	20	84.5±19.3	1.8	79.7±18.3	87.0±23.1	82.1±17.0	86.6±18.8	87.1±19.2
2,4,5,2′,5′	0.942	0.1	14	82.0±17.4	1.8	80.0±11.7	81.7±34.2	80.4±12.4	83.9±14.2	83.8±14.7
2,4,5,2′,3′	1.012	0.1	11	53.1±12.5	2.0		60.2±10.4	51.7± 5.7	50.5± 5.7	50.5±28.3
2,3,4,2′,5′	1.033	0.1	20	88.5± 8.2	2.0	84.2± 9.4	88.8± 9.0	89.0± 7.8	89.6± 6.8	91.1± 7.9

ods originally developed for packed columns. In addition the recoveries were very variable.

Extraction

We tested hexane:diethylether (1:1, vol/vol), hexane and hexane:acetone (1:1, vol/vol) as solvents for the extraction. In line with earlier findings (*5,7,8,14*), hexane:diethyl ether gave the best recovery for PCB isomers.

The use of the sulfuric acid was an efficient first step in removing coextracting compounds. The problem of negative peaks—probably caused by fatty acids—was solved by the use of sulfuric acid. The optimal amount of sulfuric acid was 2.5 mL; use of smaller amounts sometimes resulted in yellow concentrates, which invariably gave bad chromatograms.

Sodium sulfate is generally used to dry an organic solvent. Even small smounts of water decreased the sensitivity and incerased the baseline noise in PCBs analysis.

A variety of disturbing chemicals are removed by the silica columns. Commercial columns such as Sep-Pak Silica, Sep-Pak Florisil (Waters Associates, Milford, MA,), Chrompack silica (Chrompack, Middelburg, The Netherlands), Baker silica, Extrelut silica were tested. We also tested deactivated silica (0.5 g and 3.0 g) and silica activated at +140°C or at +200°C overnight. Bond Elut silica columns give the best and most constant recovery and least contaminants of their own (after prewashing as described).

The concentration of the hexane phase was performed at room temperature. Higher temperatures were also tested but especially the PCBs compounds with low degree of chlorination seemed to be lost at temperatures higher than 30°C.

The detector response toward different PCB isomers varies over 50-fold (see Table 1). Therefore, it is evident that a quantitation based on isomer-specific responses of the detector must give more accurate results than methods ignoring this difference (*15*). The selection of the PCB congeners used as standards was based on the frequency of the isomers in commercial products and in the nature (*2*). It should be noticed, though, that in the analysis we did not rigorously identify our peaks: PCB derivatives coeluting with a standard were quantitated as an entity; it is possible that our peaks contained more than one isomer.

Recovery and Imprecision of the Method

The recovery of the method was calculated in every series of analyses. The recovery and imprecision for the sum of the isomers in consecutive runs and at different analytical levels are presented in Figure 2. The overall recovery of the methods is 84.6 ± 11.4%. A practical limit of detection for this method is 0.1 μg/L of total PCB. Thus the method is well suited even for studies on populations not overtly exposed (see below). Strong effort was put here in verifying the recovery of PCB and correction of the results for it. However, the question of recovery cannot be fully settled: the spiked specimens may be different from authentic ones (*16*). On the other hand, currently there are no commercial quality control materials available for accuracy testing of PCB analysis in serum.

To estimate the imprecision of the method, three control pools were repeatedly analyzed with the unknowns. At a level of 1.8 μg/L (control 0), the variation coefficient was 46.7% (N = 25), at the level of 5.5 μg/L (control 1) it was 25.3% (N = 20) and at the level of 20 μg/L (control 2), 12.5% (N = 20).

Using this method the reference value of PCB in the serum has been analyzed for Finns not having any spe-

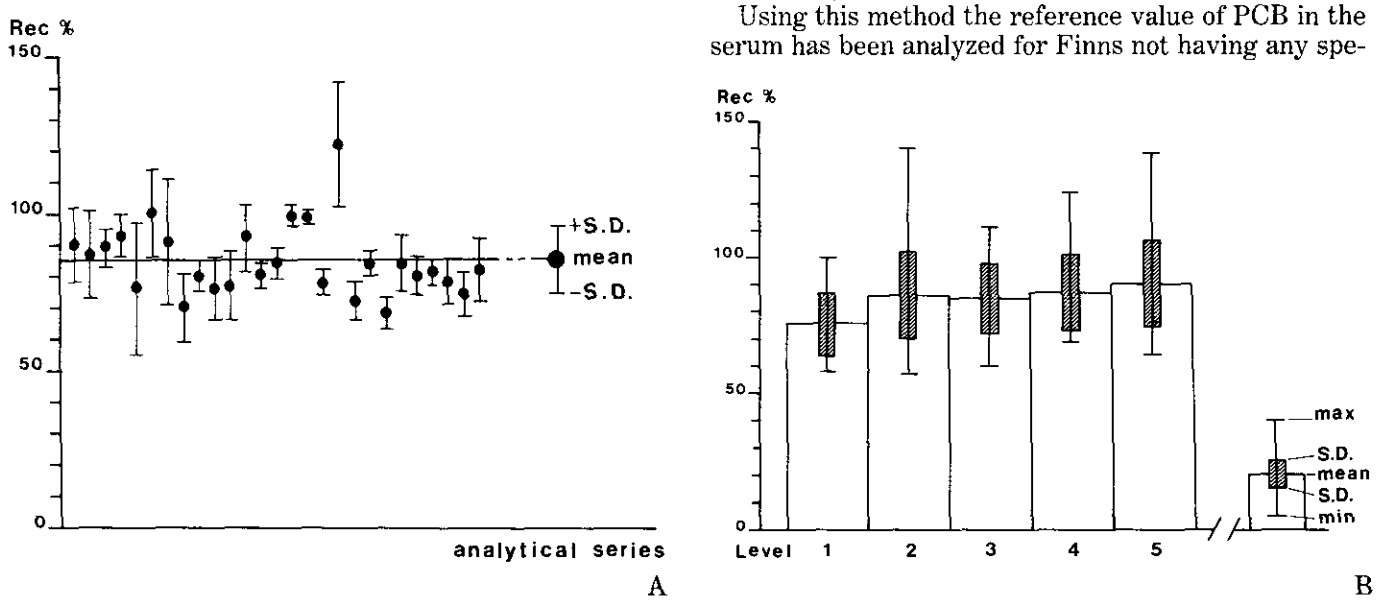

FIGURE 2. Recovery and imprecision of the method: (*a*) recovery and coefficient of variation of consecutive analytical series; (*b*) recovery and imprecision of the method calculated from sum concentrations at various concentration levels (mean ± SD, range): level 1 = 25 μg/L, level 2 = 50 μg/L, level 3 = 75 μg/L, level 4 = 100 μg/L and level 5 = 125 μg/L.

cial exposure to PCBs. For 47 analyzed samples the geometric mean ± SD of the S-PCBs is 1.2 ± 0.6 μg/L. In this small population the frequency distribution of PCB levels in serum seems lognormal. Based on such distribution and 99% limit, a practical reference upper limit of 3 μg/L may be obtained.

This method is used in our laboratory for routine analysis of the PCB in serum. Most of the samples are different accidents involving capacitors containing PCBs (leaks, fires, explosions). The serum PCB levels have varied from <3 μg/L up to 50 μg/L after various accidents.

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